LETTER

Reply to Piper et al.: *Drosophila* dietary restriction—Does it hold water?

We have shown that ad libitum water abolishes life-span extension in classical paradigms of *Drosophila* dietary restriction (DR) (1). These findings were replicated in both genders, on multiple diets, genotypes, and enclosures, making our studies generally relevant to the fly community (1). Using different conditions, Piper et al. (2) fail to reproduce one of our experiments. The authors correctly point out that one of our regimes inadvertently contained more sucrose than their proposed diet (2, 3). However, for unclear reasons, Piper et al. also used a different setup, in which water surface is limited (as opposed to our unrestricted source). The authors attempt to validate their method indirectly by rescuing a different stressor. Naturally, different insults may be rescued by different amounts of water. Thus, their setup may allow enough drinking to rescue one, but not the other, stressor. Unfortunately, Piper et al. did not measure water consumption. Thus, the contradictory results may arise from the differences in either medium composition—as Piper et al. suggest—or water delivery method. To categorically settle the issue, longevity should be assayed in the experimental conditions found to abolish the DR effect. Given the unsettling implications of our work for the field, each laboratory paradigm should be rigorously tested.

The statistical point raised by Piper et al. (2) is tangential to the central issue: life-span extension in classical DR paradigms is abolished in the presence of ad libitum water (1). Because this is not the case in rodents, our results strongly indicate that classical fly paradigms are not a relevant model of mammalian DR, and discoveries originating from these systems are of uncertain relevance and should be retested in water-independent paradigms. The mechanism of life-span extension on the particular diet at stake is a minor issue to both our work and the community. This regime was established only in 2007 (3) and differs substantially from any of the paradigms shown to affect *Drosophila* life span over the past two decades. Moreover, its effects on life span vary widely (2–4).

Our results also support the ground-breaking work of Lee et al. (5), which indicates that the protein:carbohydrate ratio (P:C) is the key dietary determinant of fly life span. In light of these findings, an ideal DR paradigm should modulate the P:C ratio, whereas *Drosophila* DR has relied almost exclusively on whole-medium dilution, holding the P:C ratio constant in ranges that entirely miss optimal life span. The recently proposed regime (3) represents a modest improvement over classical conditions because it varies yeast, but not sucrose, levels, thereby modulating the P:C ratio. However, the change is minimal—from 1:1.3 (fully fed) to 1:1.9 (DR) [assuming that Brewer's yeast contains 45% protein and 35% carbohydrate (3)] and either diet is far from reaching the optimal 1:16 (1, 5). The current knowledge strongly indicates that these conditions are inadequate. If *Drosophila* is to serve as a model for mammalian DR, future work should (*i*) vary the P:C ratio within appropriate ranges, (*ii*) verify that hydration is not a factor, and (*iii*) quantify feeding rate.

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